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Chapter 33

Mariculture of coral colonies for the public aquarium sector

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ABSTRACT

Coral-reef aquaria are some of the most colorful and attractive displays in zoos and public aquaria. Used either for general exhibition or for educational purposes, such aquaria should display a high number of coral colonies from diverse coral species and morphologies. Whereas periodic replacement of dead and diseased coral colonies in an already established aquarium can be achieved by propagation (either sexually or asexually) of the existing coral stocks, starting an entirely new display aquarium system requires an agricultural approach. Furthermore, because coral reefs around the world are declining, trading in wild corals is restricted by CITES regulations. Previously, we developed protocols for large-scale *ex-situ* and *in-situ* farming of thousands of coral colonies in protected nurseries. The farmed colonies originated from more than 15 different coral species, mostly of branching forms, ranging from minute ramets ("nubbins" containing few polyps only) to 3-5 cm long ramets ("fragments"). Survivorship of nubbins and fragments in both nurseries was very high, only 10-13 % of farmed ramets died. Here we present results obtained with branching corals and initial results on massive and encrusting coral species, revealing the applicability of the developed methodologies for mass production of coral colonies.

INTRODUCTION

Coral reefs are complex biological structures displaying extreme diversity of color morphs and species compositions. Coral reef aquaria reflect this biodiversity and represent one of the most attractive displays in zoos and public aquaria. Coral reefs biodiversity condensed into miniature aquaria dazzle visitors with its kaleidoscope of shapes, colors and biological interactions. Establishing new coral reef aquaria faces the initial problems of how and from where to get sufficient quantities of coral colonies for the exhibition. Documenting the continuous decline of coral reefs worldwide (Wilkinson, 2004), further kindled public attention to coral reefs' biology and ecology, in general, and to hard corals, the foundation reef builders of any tropical coral reef, in particular. The vast majority of supply for ornamental species, including reef corals, originates from the wild (Green and Shirley 1999). The devastating impacts of the fast developing trade of marine reef aquariums (Hodgson, 1999), forced CITES to issue more stringent regulations on shipping harvested corals from the reefs (Harriott, 2003). It is clear that while periodic replacement of dead and diseased coral colonies in an already established aquarium can be achieved by small-scale propagation (either sexually or asexually) of the existing coral stocks, starting an entirely new display aquarium system requires an agricultural approach. To alleviate the shortage of live material for marine tropical exhibitions and the reef aquarium market, it is suggested (Bruckner, 2000) to construct coral nurseries and reef fish hatcheries that will supply the demand from *ex-situ* or *in-situ* facilities without harming natural habitats.

Red Sea Corals Ltd. (Kibbutz Saar 22805, Israel) is engaged in the development of reef restoration methodologies. Using this knowledge (Shafir *et al*., 2006a,b), it is suggested to develop *in-situ* and *ex-situ* coral nurseries where thousands of coral colonies are farmed for the marine aquarium market. The

demand for large numbers of maricultured coral colonies for restoration and the methodologies developed for it (Rinkevich, 2006) can also satisfy the demand of the public aquaria sector and the ornamental trade.

The process of coral asexual propagation by fragmentation produces genetically identical colonies from a specific coral genet. Large corals ramets, usually 1-5 cm are referred to as fragments, while small corals ramets, 0.5-1 cm diameter, are referred to as nubbins (Shafir *et al*., 2001). This is a well established procedure for mariculturing large numbers of colonies from branching coral species (Shafir *et al*., 2006a,b), for the purpose of reef restoration. The same approach could be applied to the marine aquarium market (Shafir *et al*., 2001). However, there are significant differences between coral nurseries for restoration and coral nurseries for the aquarium trade. Restoration strategies emphasize raising as many coral species as possible, both fast and slow growing species, and those easy and difficult to farm. The public aquarium sector can be satisfied with a limited number of coral species for display, mainly those that are more appealing to the visitors and economic to farm. Additionally, restoration projects aim to preserve high genetic variability (Amar and Rinkevich, 2007), whereas the aquarium market naturally favors fast growing coral genotypes (at the expense of slow growing genotypes) that readily reproduce asexually and therefore only specific genotypes are selected. Reducing the numbers of coral species and genotypic diversity within those species also serves scientific research needs where assays are applied to clonal line of a limited number of model coral species (Shafir *et al*., 2006c).

In view of the above rationale, this chapter presents protocols for producing large quantities of coral colonies from fragments and nubbins, with an eye to farming of coral colonies suitable for any size of coral reef aquarium, for exhibition, education, and research applications.

MATERIAL AND METHODS

The protocols described below are for asexually propagation of corals in two ramet sizes: fragments and nubbins.

Protocols:

1. *Coral fragments from a branching species*

Use an electrician's wire-cutter to prune small branches, 2-5 cm long, from donor colonies (work can be preformed out of water, although it is preferable to work with both hands submerged in the aquarium). It is important to keep the ratio between the base of the ramet and its height as small as possible (2-3 height/diameter ratio), i.e., thinner branches are shorter. This ratio reduces the forces on the attached area and detachment. Use branches from all parts of the colony, tip, mid and bases.

Wash fragments by shaking them under seawater to get rid of debris of small tissues and skeleton.

Attach branch fragments to substrate by either gluing them to heads of plastic pins or inserting coral fragments into plastic anchors. Work should be performed out of water and gloves should be used to minimize damages to coral tissues.

a. Use plastic pins (such as provided by Red Sea Corals LTD., Israel; 9 cm long, 0.3-0.8 cm wide leg with a 2 cm diameter ''head'', Figure 2a) for all source coral material. Place the cut area on a paper towel and dry the skeleton. Place a small drop of Super glue 3 (e.g., Loctite® super glue, Ireland) on the plastic pinhead, the glue drop should be same size as the cut fragment area. Stick the cut area onto the glue and hold for few seconds (Figure 1a). Check that the fragment is attached to the substrate and then insert it in a seawater tank (Figure 1b). Place the pins with the corals on a plastic net inside a seawater tank (Shafir *et al*., 2006a,b). The same may apply for massive and encrusting coral species.

b. Use plastic anchors of various sizes, of the available common brands (Figure 1c). Add a small drop of Super glue 3 to the plastic anchor and then firmly insert a coral branch into a suitable size plastic anchor, check attachment and place it within the plastic net (Figure 1c).

Plastic anchors are suitable primarily for thinner and longer branching corals. The procedure using plastic pins is more time consuming than the one using plastic anchors. Branches attached to plastic pins are prone to breaking off from substrates, especially if handled by inexperienced workers. The plastic anchor procedure, in which

dead coral skeletons are inserted into the anchors, is less prone to being detached.

In both protocols, branches grow over the plastic base and onto the surrounding substrates within 20 to 40 days (Figure 1d). Plastic pins can be used with all corals species, including massive and encrusting forms, or large fragments originating from bases of branching species.

2. *Coral nubbins from branching species*.

Nubbins are minute fragments consisting of one to several polyps of approx. 0.25 cm^2 to 0.5 cm^2, depending on the coral species. We developed and adjusted nubbin protocols for several coral species (Shafir *et al*., 2006c).

Cutting a coral fragment into nubbins

This procedure can be performed on fragments submerged in seawater or exposed to the air. Wear gloves to minimize damage to coral tissues. Use an electrician's wire-cutter to cut medium (3-5 cm) ramets from a donor coral colony (we routinely use *Stylophora pistillata* and *Pocillopora damicornis;* Figure 2b). Cut each ramet into 0.5 cm wide slice fragments (Figure 2c); then, halve each slice (Figure 2d). If the slice is large enough, it can be divided further into four quarters (4 nubbins Figure 2e). Leave the newly formed nubbins in the seawater tank for a few minutes and let the water wash away debris of small tissue and skeleton.

Attach nubbins to substrates

This step should be performed out of water. Place the plastic pin vertically in a holding net and add a small drop of super-glue onto the plastic pinhead (Figure 2g). Take a nubbin out of the water with forceps and place it

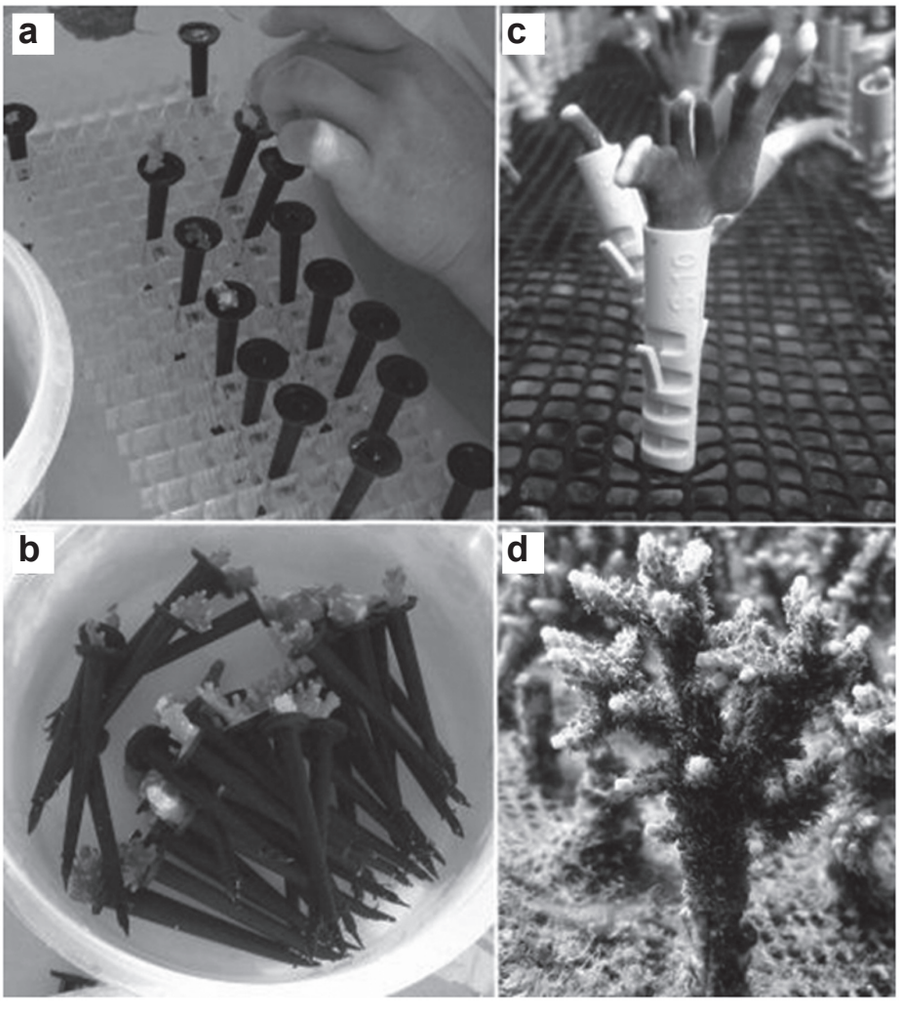

Figure 1: Plastic pin and plastic anchor procedures for coral propagation (a) gluing fragments of Pocillopora damicornis *onto plastic pins; (b) freshly prepared glued nubbins in a plastic container before transferring them to the holding nets; (c) a small branch of the hydrocoral* Millepora dicotoma *inserted into a plastic anchor; (d) tissue growth of* Acropora variabilis *over the plastic anchor.*

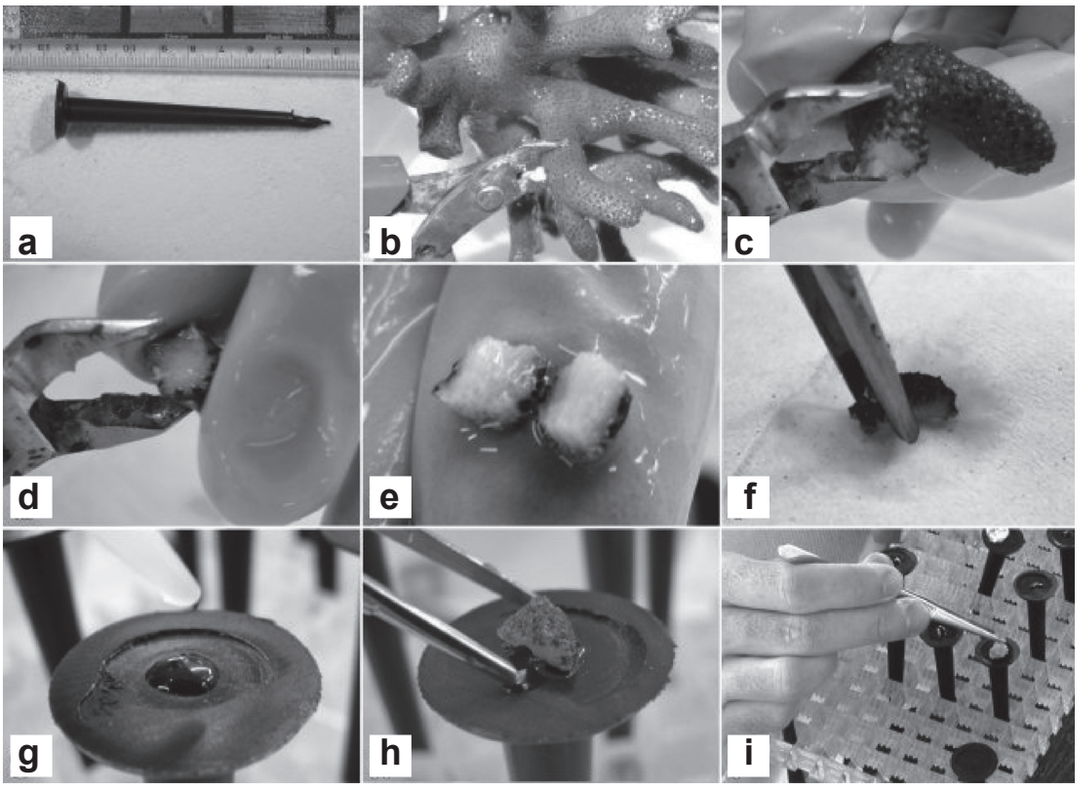

Figure 2: Illustrated description of the protocol for generating coral nubbins (see text for details)

with the exposed skeleton side on a paper towel to absorb excess water (Figure 2f). Using forceps, place the nubbin's exposed skeleton onto a drop of super-glue (Figure 2h,i). It is not necessary to press the nubbin against the plastic. Repeat this procedure with more nubbins. After ca. 10 seconds, check attachment by gently stroking the plastic pin against the table. Re-fix detached nubbins. Place the substrate with the nubbins into a holding device and immerse it in a seawater tank. Nubbins should be exposed to air for no more than one minute during this procedure. Transfer the nubbins into your coral husbandry system. Maintenance conditions are described in Shafir *et al*. (2001).

RESULTS AND DISCUSSION

The protocols for fragmenting coral colonies into small branches and nubbins enabled us to develop tens of thousands of nubbins and coral fragments from 16 species (Table 1), followed by mariculture of thousand of corals in a mid underwater nursery (Shafir *et al*., 2006a,b) and within *in situ* facilities (Shafir *et al*., 2001). While the basic methodology is the same, preparations of branch fragments and nubbins slightly differ for each of coral species (*Stylophora*, *Pocillopora, Acropora, Seriatopora*, *Porites* and *Favia*), requiring a flexible approach. The thickness of the used fragments and other factors like excess mucus secretion by some coral species (*Acropora pharaonis*; Shafir *et al*., 2006a) or survival rates, call for species-specific adjustments.

We found that the best way to prune corals is with an electrician's wire-cutter: larger cutters for thick branches and smaller for the thin branches and nubbins (Figure 2b-d). It is advisable to cut the corals along the skeletal growth lines, where cuts leave the peripheral coral tissue on the fragments firmly attached to the skeleton, promoting faster and more successful healing and regeneration. Using disk blade or chisel for this purpose has increased damages to the fragments' tissues. However, when working with massive coral forms, a chisel is used to produce at first small pieces that then divided further with a cutter. Attaching a coral fragment or a nubbin to the substrate is time consuming. Using plastic anchors of various sizes could accommodate various sizes of branching coral fragments. Use of commercially available superglues is highly efficient, savings both time and labor, especially for attaching nubbins. Larger fragments (over 5 cm high) need stronger epoxy cements that form larger areas of contact with substratum. Epoxy cements require considerably longer

curing periods than superglue but are better for holding large coral fragments.

Results reveal a significant variability in growth rates and survivorship between different genotypes of a specific coral species (*Pocillopora damicornis*, Shafir *et al*., 2006a). The suitability of a coral genet for the public aquarium sector can be therefore tested before a coral colony is pruned to hundreds of fragments by testing growth rates of a few nubbins from potential donor colonies. Corals species differ in their ability to regenerate, including their ability to develop horizontal tissue and skeletal material that attach them to the substrate (Table 1). On the use of plastic tips/anchors to attach corals allows aquarists to easily transplanted coral colonies by drilling a hole into the substrate and inserting the tissue-free lower part of the device into the hole. Corals can be attached to any structure, even on vertical walls.

By using the nubbin size fragments, we found (Shafir, unpublished) that hundreds of new coral colonies can be generated from a relatively small (10-15 cm diameter) branching coral colony. A single experienced person is able to cut and prepare about 100 nubbins in 30-40 min. Loss of coral material is negligible as nubbins can be obtained from all parts of the coral colony, including coral branches and coral base. It is also evident that there is a need to establish *in-situ* and *ex-situ* methodologies for farming large numbers of coral colonies, mainly for new large reef aquaria that need thousands of corals colonies in a short time.

We recommend the use of nubbins as the major source material for the public aquarium sector, which is also environmentally friendly. Nubbins can be easily obtained from collected broken fragments (corals of opportunity) that are doomed to die on the sea or aquarium floor**.** A small branch fragment can yield 20-30 nubbins with high survival rate (>85 %; Shafir *et al*., 2006c). Repeated pruning of daughter colonies may provide large stocks of ramets that are no longer considered to be wild collected material by CITES (www1), this allowing easier coral material exchange between aquaria. The corals that are adapted to the aquarium conditions, may live longer reducing the need for additional material from the wild. Repeated pruning suites the public and private aquarium market, reef restoration fragmentation deal with higher genetic variability.

Table 1: Summary of results for coral fragmentation and nursery farming characteristic to different tested coral species. Nubbins' application: + average, > 50 % survived, ++ good, > 75% survived; +++ excellent, > 90 % survived. Growth rates: + low growth rates, < X20 size augmentation/year, ++ medium growth rates, X20-50 size augmentation/year, +++fast growth rates, >X100 size augmentation/year. Self-attachment (nubbin's and fragments that spread on the substrate): - no self-attachment, + low self-attachment, 20 %-50 %, ++ medium self-attachment 50 %-80 % and +++ high self-attachment > 80 % (Shafir et al*., 2006a,b,c and unpublished results).*

Coral species	Fragment size (cm)	Number	Nubbins' application	Growth rates	Self attachment	Mortality rates (%) assayed at day
Acropora eurystoma	1-5	687	+	+++	+++	12%, day 144
Acropora hemprichii	2-3	32	+	++	+++	0%, day 222
Acropora humilis	3-5	18	+	++	++	6% ,day 292
Acropora lamarcki	3-5	30	+	++	++	3%, day 292
Acropora millepora	2-4	60	+	++	++	14%, day 180
Acropora pharaonis	3-5	527	+	+++	+++	10% ,day 144
Acropora squarrosa	2-3	18	+	++	++	6%, day 292
Acropora valida	1-3	1054	+	+++	+++	12%, day 144
Acropora variabilis	3-5	36	+	+++	+++	3%, day 292
Favia favus	1-2	30	+	+	+	50%, day 128
Millepora dichotoma	2-3	156	++	+++	+++	4%, day 292
Pocillopora damicornis	1-5	>5000	+++	+++	+++	5-15%, day >300
Porites sp.	0.5-1	30	+++	+	+++	5%, day >300
Seriatopora hystrix	1-5	48	+++	+++	-	0%, day 292
Stylophora pistillata	1-5	>5000	+++	+++	+++	5-15%, day >300
Turbinaria sp.	1-3	50	+	+	-	2% ,day >300

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INTERNET RESOURCES

www1. www.CITES.org